Enzymatic Decolourization of Textile Dyeing Wastewater by the White Rot Fungus Phanerochaete Chrysosporium

Kannan Pakshirajan[1]*, P. Radhika[2]

[1]*Department of Biotechnology, Indian Institute of Technology Guwahati, Guwahati 781039, Assam, India

[2]Faculty of Health and medical science, University of Surrey, Guildford, Surrey GU2 7XH., United Kingdom

E-mail: pakshi@iitg.ernet.in, r.pothi@surrey.ac.uk

Abstract

Effluent from textile industries often contains toxic dyes and other chemicals, which make it become difficult to reuse such wastewaters. The present study was aimed at decolourizing wastewater collected from a textile dyeing industry using the white rot fungus *Phanerochaete chrysosporium* in batch shake flasks. The wastewater was initially characterized for the parameters chemical oxygen demand (COD), color index, total suspended solids, pH, heavy metals and alkalinity. Wastewater decolourization using *P. chrysosporium* was then investigated under different conditions such as, dilution with water and with the fungal growth media at 1:1 and 1:3 proportions. During the wastewater decolourization, high enzyme activities of lignin peroxidase and manganese peroxidase produced by the fungus were observed at 84 and 96 *h*, respectively, particularly in the experimental flasks containing the wastewater and the fungal growth media. Laccase activity by the fungus was, however, absent in the experiments. This study clearly demonstrated the role of lignolytic enzymes in decolourization of the wastewater.

Keywords

Phanerochaete Chrysosporium; Lignin Peroxidase; Manganese Peroxidase; Textile Dyeing Wastewater; Decolourization

Introduction

Textile industries consume substantial volume of water and chemicals for wet processing of textiles and other unit operations. A variety of chemicals are also used for scouring, bleaching, dyeing, printing and finishing [1], which range from inorganic compounds and elements to polymers and organic products. More than 8000 chemical products are found associated with the dyeing process [2] and over 100,000 commercially available dyes exist with over 7×10^5 metric tons of dyestuff produced annually [3]. These dyes include several structural varieties such as acidic, reactive, basic, azo, di-azo, anthraquinone based and metal complex dyes, and azo dyes contribute the largest of these group of dyes used in the textile dyeing industry.

It has been shown that azo- and nitro-compounds in textile dyeing wastewaters are reduced in sediments [4] and in the intestinal environment [5], resulting in the regeneration of the parent toxic amines. Anthraquinone-based dyes are the most resistant to degradation due to their fused aromatic structure and hence wastewater containing these dyes remains coloured for long periods of time. Basic dyes have high brilliance and therefore possess high color intensity, making them difficult to decolorize, whereas metal-based complex dyes, such as chromium-based dyes, can lead to the release of potentially carcinogenic chromium into water supplies. Some disperse dyes have also been shown to have a tendency to bio-accumulate [6], and both algae and higher plants exposed to textile dyeing effluents have been reported to contain high levels of heavy-metals.

Several physico-chemical methods have been tested to treat textile dyeing wastewater, but these suffer from one or more serious drawbacks such as inefficient colour removal, requirement of costly chemicals and reagents, high operation and maintenance cost, secondary sludge generation problem etc [7, 8]. On the other hand, biological methods are proving more effective than conventional methods in decolourization of such wastewaters mainly by the process of biodegradation or biomineralization of dyes present in the wastewater. In particular, the white rot fungus *Phanerochaete chrysosporium* has been extensively studied for wastewater decolourization owing to its non-specific extracellular oxidative enzyme system constituted by lignin peroxidase (LiP), manganese

peroxidase (MnP) and laccase. In the nature, the fungus uses LiP to degrade complex lignin for deriving its nutrition [9]. Laccases are multi-copper phenol oxidases that can decolorize dyes through a highly non-specific free radical mechanism by forming phenolic compounds, thereby avoiding the formation of toxic aromatic amines [10]. MnP is a heme containing enzyme known to catalyse the oxidation of Mn^{2+} to Mn^{3+}, which in turn can oxidize a variety of phenolic substrates, including lignin [11]. In fact, by virtue of these lignolytic enzymes produced by this fungus, it has been shown to degrade a wide variety of pollutants ranging from simple phenolic compounds to polycyclic aromatic compounds and other xenobiotics [12]. However, the role of this fungus and its enzyme system in decolourization of real textile dyeing wastewater has not been sufficiently addressed in the literature. This is particularly important considering the fact that such wastewaters contain a complex mixture of dyes and other recalcitrant chemicals. Hence, this study was aimed at characterization and decolourization of wastewater from a textile dyeing industry using the white rot fungus *P. chrysosporium*. The role of the lignolytic enzymes produced by the fungus was examined on the removal of colour and COD from the wastewater.

Materials and methods

Chemicals

Veratryl alcohol (3,4-dimethoxybenzyl alcohol, 96% pure) and 'ABTS (2,2 -Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) were purchased from Sigma (St. Louis, Mo, USA); all other chemicals and solvents were purchased from either High Media, Mumbai (India), SRL (India) or Merck (India), which were all of GR grade.

Microorganism and media composition

The fungus *P. chrysosporium* MTCC 787, used in this study was procured from IMTECH, Chandigarh, India, and was maintained at 25 ºC on potato dextrose agar (PDA) slants. For spore production, the slants were incubated at 39 ºC for 2 to 5 *d* in medium containing glucose: 10 g/L, malt extract: 10 g/L, peptone: 2 g/L, yeast extract: 2 g/L, asparagine: 1 g/L, KH_2PO_4: 2 g/L, $MgSO_4 \cdot 7H_2O$: 1 g/L, thiamin-HCl: 1 mg/L and agar: 20 g/L [13]. The media used for decolourization of the wastewater composed of basal medium (KH_2PO_4, 20 g/L; $MgSO_4$, 5 g/L; $CaCl_2$, 1 g/L), trace elements ($MgSO_4$, 3 g/L; $MnSO_4$, 0.5 g/L; NaCl, 1 g/l; $FeSO_4 \cdot 7H_2O$, 0.1 g/L; $CoCl_2$, 0.1 g/l; $ZnSO_4 \cdot 7H_2O$, 0.1 g/L; $CuSO_4$, 0.1 g/L; $AlK(SO_4)_2 \cdot 12H_2O$, 10 mg/L; H_3BO_3, 10 mg/L; $Na_2MoO_4 \cdot 2H_2O$, 10 mg/L; nitrilotriacetate, 1.5 g/L) and other ingredients - glucose, 100 g/L; 2,2-dimethylsuccinate, 0.1 M (pH 4.2); thiamine, 100 mg/L (filter sterilized); veratryl alcohol, 4 mM stock (filter sterilized) and NH_4Cl, 4.68 g/l [14].

Decolourization experiment

Wastewater decolourization experiments using the fungus were performed with coloured industrial effluent collected from a textile dyeing industry located in Punjab, India. The wastewater was initially characterized for chemical oxygen demand (COD), pH, alkalinity, total suspended solids, total dissolved solids, heavy metals and other parameters as per the Standard Methods [15]. For performing the decolourization experiments, four duplicate sets of Erlenmeyer flasks (250 ml) with 100 ml working volume each were taken. While the first set of flasks (A) contained only the wastewater, the second set (B) contained wastewater diluted with an equal volume of demineralized water. The third (C) and fourth (D) sets of flasks contained wastewater diluted with the decolourization media at proportions 1:1 and 1:3, respectively. These flasks were inoculated with *P. chrysosporium* spores (OD 650 nm = 0.5 $\approx$ 2.5×10^6 spores/ml) followed by incubation in a rotating orbital incubator shaker set at 180 rpm and 39°C. Flasks without the fungal culture served as control in the experiments. Samples (3 ml each) were collected at specific time intervals for the analyses of enzymes, glucose, COD and color. Whereas 1 ml of sample was collected every 12 h for analysis of LiP, MnP and laccasse, for glucose analysis 1 ml sample was collected every 4 h. For the analyses of COD and color, samples (1 ml each) were collected every 24 h. Prior to the analyses, the samples were centrifuged at 10,000 × g for 10 min at 4 ºC to remove the fungal biomass. Wastewater decolourization was monitored by scanning the samples from 200 nm to 800 nm using a UV-Vis spectrophotometer (Carry 100, Varian, USA) and observing the change in absorbance. Glucose estimation was carried out following the Anthrone method [16]. Results reported are average of triplicate sample analyses.

Enzyme assay

LiP activity in the samples was determined based on the oxidation of veratryl alcohol to veratrylaldehyde in

the presence of H_2O_2 [17]. One unit (U) of the enzyme was defined as the amount that converted 1 mol of veratryl alcohol to veratraldehyde per minute per ml of the culture filtrate and the enzyme activity was expressed as U/L. Similarly, MnP activity was assayed based on the oxidation of 1mM $MnSO_4$ in sodium malonate buffer in the presence of H_2O_2, and for laccase assay, the substrate ABTS (2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) was used [18]. All these enzyme assays were performed using a UV-Visible spectrophotometer (Carry 100, Varian, USA).

Results

Wastewater characteristics

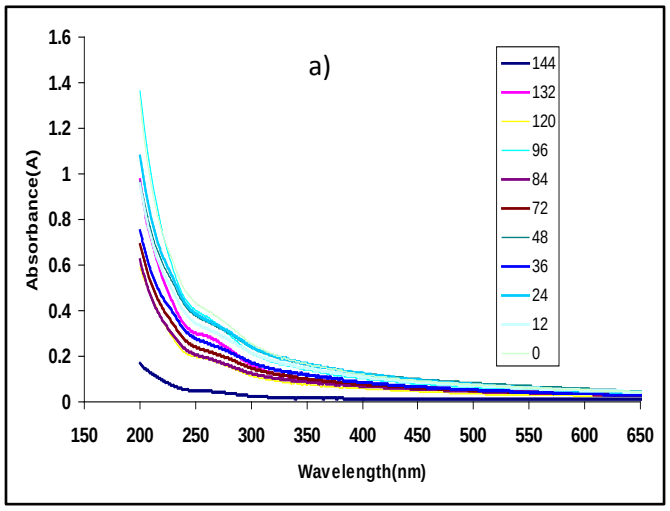
Table 1 presents the characterization results of the textile dyeing wastewater used in this study.

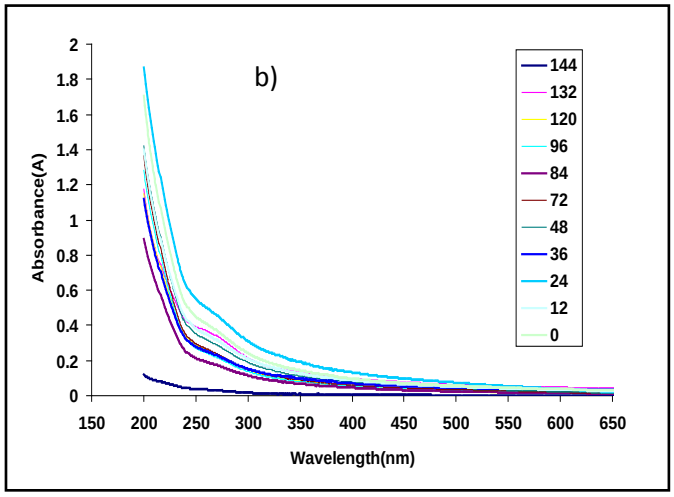
TABLE 1 COMPOSITION OF THE TEXTILE DYEING WASTEWATER USED IN THIS STUDY

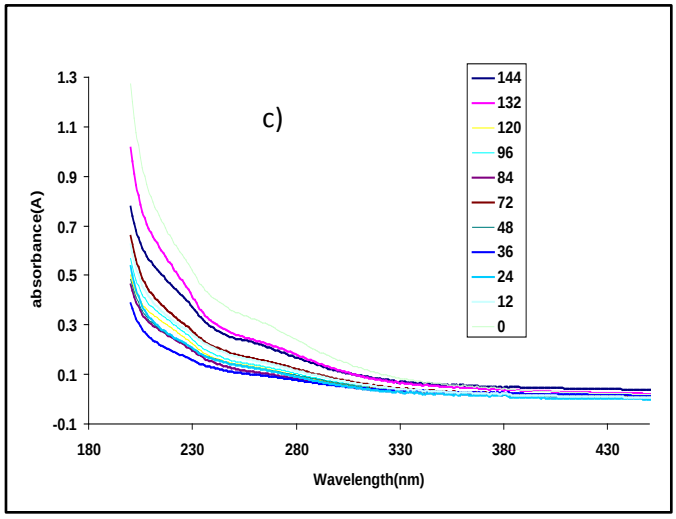
S. no	Parameter	Values (mg/L)
1	pH	9.00
2	Total suspended solids	250
3	COD	6000
4	Chromium	1.5
5	Copper	0.124
6	Iron	0.557
7	Nickel	0.121
8	Lead	0.290
9	Zinc	0.168
10	Alkalinity as carbonate and bicarbonate	Absent

Wastewater decolourization and COD removal

Absorbance change in the wastewater due to colour removal by the fungus in the different experimental flaks is depicted in Fig. 1(a-d), which indicates significant reduction in colour at the end of 6 *d* in all the flasks. From the absorbance decrease in each flask, percentage decolourization was calculated and is presented in Fig. 2. These results revealed maximum decolourization of about 85% and 93% occurred only in Flasks C and D that contained wastewater mixed with the fungal growth media at 1:1 and 1:3 ratios, respectively (Fig. 2). In the literature, similar role of the media have been demonstrated for dye decolourization by the fungus [19].







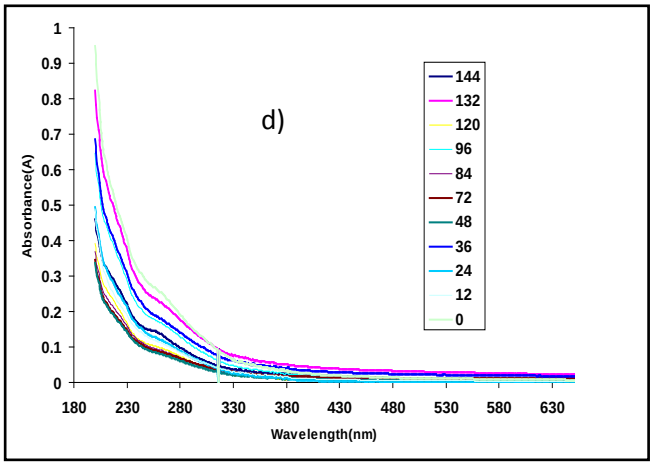


FIG. 1 CHANGE IN ABSORBANCE DUE TO COLOUR REMOVAL BY *P. CHRYSOSPORIUM* IN THE DIFFERENT EXPERIMENTAL FLASKS: A) FLASK A, B) FLASK B, C) FLASK C AND D) FLASK D. (UNIT FOR LEGEND ENTRIES IS H)

Fig. 3 shows reduction in wastewater COD by *P. chrysosporium* in the four different experimental flasks. Initial COD in the flasks varied depending upon whether the wastewater was undiluted or mixed with water or with the fungal growth media containing glucose at different proportions. It could be seen from Fig. 3 that in Flasks C and D, which contained wastewater mixed with the fungal growth media at 1:1 and 1:3 proportions, respectively, reduction in COD was significant. The COD removal by the fungus was also very high in flask with wastewater diluted with only water, i.e. in Flask B. On the other hand, COD removal in the flask containing only wastewater remained very low.

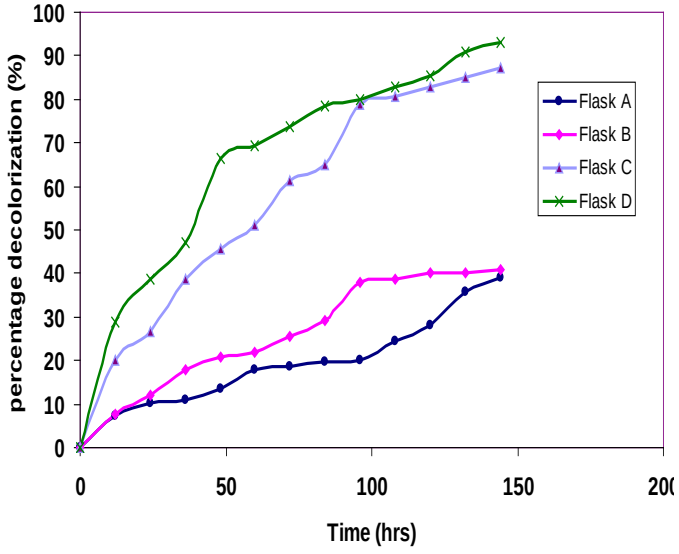


FIG. 2 WASTEWATER DECOLOURIZATION EFFICIENCY ACHIEVED UNDER DIFFERENT EXPERIMENTAL CONDITIONS

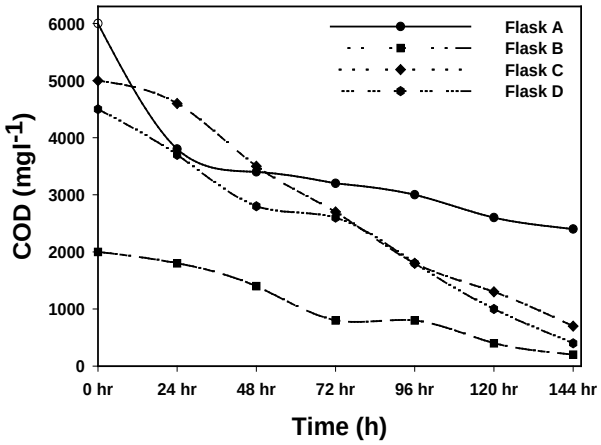


FIG. 3 WASTEWATER COD REMOVAL BY *P. CHRYSOSPORIUM* IN THE DIFFERENT EXPERIMENTAL FLASKS

Glucose estimation in the samples collected from Flasks C and D was also performed as the wastewater in these flasks was mixed with the fungal growth media at different proportion. Fig. 4 shows that glucose in both the flasks was completely consumed by the fungus within 36 h of culture.

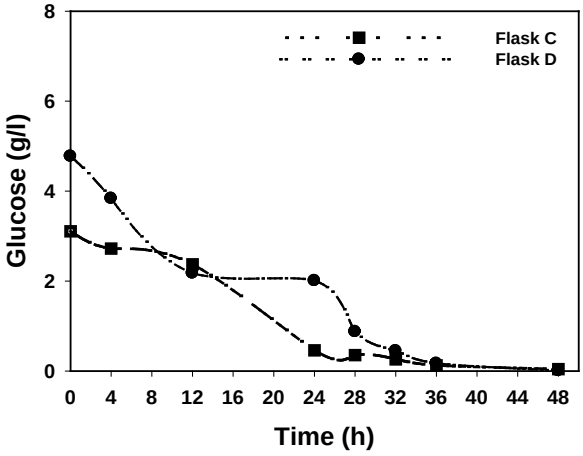
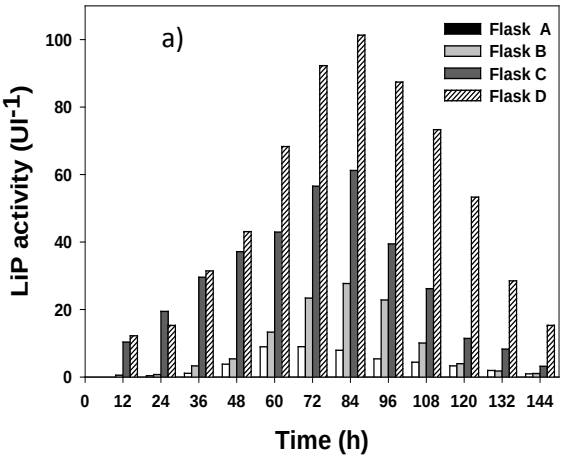


FIG. 4 GLUCOSE UTILIZATION BY *P. CHRYSOSPOIRUM* IN FLASKS C AND D

Enzyme activity



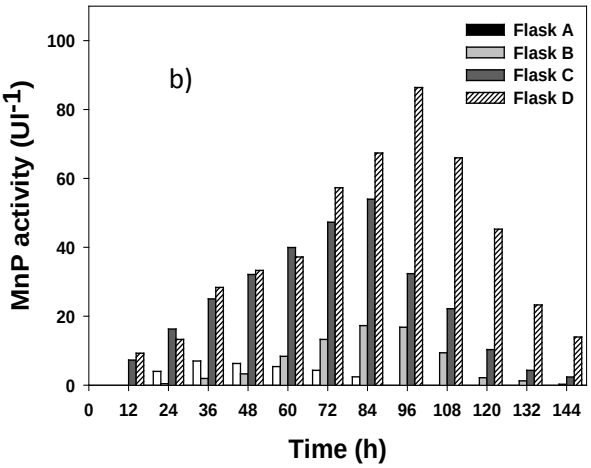


FIG. 5 ENZYME ACTIVITY DUE TO *P. CHRYSOSPORIUM* IN THE DIFFERENT FLASKS: A) LIP AND B) MNP

LiP and MnP activity obtained in the different flasks containing the wastewater is shown in Fig. 5, which indicates that maximum enzyme activity (both LiP and MnP) was obtained with Flask D in which the wastewater was diluted three times with the fungal growth media. Whereas maximum LiP activity obtained was 86.38 U/L at 96 h, in case of MnP it was 101.28 U/L at 96 h culture. The enzyme activity values obtained with Flask C were less than those observed with Flask D. On the other hand, negligible activity of these enzymes was observed with the other two flasks: Flasks A and B (Fig. 5).

Discussion

Wastewater characteristics

Characterization of the industrial wastewater (Table 1) showed that the total suspended solids exceeded the permissible limit of 5 mg/L. Carbonate and bicarbonate alkalinity was found to be absent in the wastewater (Table 1), which when presented can act as carbon source for algal growth leading to an increase in pH as high as 9-10 due to accumulation of hydroxide ions [20]. Thus, although the wastewater showed no alkalinity due to carbonates and bicarbonate, high pH of the wastewater could be due to the hydroxide ions present in it, which needs further analysis to confirm. Heavy metal content of the wastewater was within the permissible levels.

Influence of media addition on wastewater decolourization

Initial absorbance due to the wastewater (in Flask A) was very high and corresponded to 1.8 (Fig. 1a) probably due to the presence of highly coloured compounds such as dyes. In case of the other flasks, the initial absorbance was less due to dilution of the wastewater with water or the fungal growth media (Fig. 1b, 1c, 1d). A maximum decrease in the color (40 %) solely due to the dilution effect was seen in Flask D which contained 1:3 ratio of the wastewater to decolourization media. However, in all the flasks wastewater decolourization occurred during the 6 *d* of batch culture with the fungus that resulted in decrease in its absorbance with maximum decolourization occurring in Flasks C and D (Fig 1 c-d and Fig. 2). COD reduction in the wastewater also followed a similar trend (Fig. 3) which revealed that the decolourization occurred due to degradation of coloured compounds and other organics present in the wastewater.

The results of wastewater decolourization and COD removal were also linked with the activities of LiP and MnP by the fungus. At the beginning of the batch culture, enzyme activity was very low in all the four experimental flasks containing the wastewaters (Fig. 5). But, over the period of the fungal culture, the enzyme activity increased particularly in Flasks C and D containing the fungal growth media in addition to the wastewater, which therefore resulted in a very high decolourization efficiency and COD removal from the wastewater. Further, a simple comparison of glucose utilization (Fig. 4) in Flasks C and D with the enzyme activities (Fig. 5) reveals that glucose along with other nutrients present was completely consumed by the fungus within 36-48 *h* for its growth and resulted in very high enzyme activity leading to effective decolourization of the wastewater. However, during the time the glucose was being consumed, the enzyme activity was still less than the maximum, which is due to the fact that the fungus produces these enzymes only under carbon and nitrogen limitation conditions [21]. Thus it is clear that maximum decolourization of the wastewater occurred due to high enzyme activity by the fungus. In addition, enzyme activity by *P. chrysosporium* and wastewater decolourization efficiency of up to 40 % even without supplementation with the media suggests the very good potential of the white rot fungus in treatment of textile dyeing wastewater.

Comparison of the wastewater decolourization efficiency with literature

Table 2 compares the wastewater decolourization efficiency obtained in this study with those reported in the literature, which reveals that the value obtained is

superior compared to other microorganisms treating either synthetic or real industry wastewater.

TABLE 2 COMPARISON OF THE WASTEWATER DECOLOURIZATION EFFICIENCY WITH THE LITERATURE

Microorganism	Type of wastewater	Maximum decolourization achieved (%)	Reference
Myrothecum sp	Synthetic wastewater containing Orange II dye	91	Latif et al. (22)
Neurospora crassa	Synthetic wastewater containing Vermelho Reantl dye	91	Latif et al. (22)
Pycnoporus	Pigment plant effluent	90	Latif et al. (22)
Candida sp	Synthetic wastewater containing Procyon Black SPL dye	93.8	Latif et al. (22)
Trichoderma sp	Hardwood extraction Effluent	85	Latif et al. (22)
P. chrysosporium	Textile dyeing effluent	95	This study

Practical implications

The fungus *P. chrysosporium* investigated in this study can be used for effective removal of colour and COD from textile dyeing effluents. However, for practical applications, the process needs to be evaluated for continuous treatment of such wastewaters using a suitable reactor system. Further, investigations on its performance under different reactor operating conditions are required to establish its potential. Finally, cost-benefit analysis will be necessary for large scale applicability of the process.

Conclusion

Decolourization of textile dyeing wastewater was successfully achieved using the white rot fungus *P. chrysosporium* in batch shake flasks. The roles of LiP and MnP enzymes produced by the fungus were clearly shown on the wastewater decolourization and COD removal. Maximum activity of the enzymes was achieved under condition where the wastewater was mixed with the fungal growth media at proportion 1:3. Glucose utilization by the fungus revealed a maximum activity of the enzymes following its exhaustion in the system. This study proved the excellent potential of *P. chrysosporium* in treating textile dyeing wastewater.

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REFERENCES

[1] M.F. Mansor "Process optimization on production of lignin peroxidase of sewage treatment plant sludge in a stirred tank bioreactor and its degradation of synthetic industrial dyes," Master thesis, Faculty of Science, International Islamic University. Malaysia, 2008.

[2] R.K. Sani, W. Azmi, U.C. Banerjee, "Comparision of static and shake culture in the decolorization of textile dyes and dye effluents by *Phanerochaete chrysosporium*," Folia Microbiologica, vol. 43, pp. 85-88, 1998.

[3] I.M. Banat, P. Nigam, D. Singh, and R. Marchant, "Microbial decolorization of textile dye containing effluents: A review," Bioresource Technology, vol. 58 (3), pp. 217-227, 1996.

[4] G. McMullan, C. Meehan, A. Conneely, N. Kirby, T. Robinson, P. Nigam, I.M. Banat, R. Marchant and W.F. Smyth, "Microbial decolorization and degradation of textile dyes," Applied Microbiology and Biotechnology, vol. 56, pp. 81-87, 2001.

[5] R. Venkatadri, and R.L. Irvine, "Cultivation of Phanerochaete chrysosporium and production of lignin peroxidase in novel biofilm reactor systems: hollow fibre reactor and silicone membrane reactor," Water Research., vol. 27 (4), pp. 591-596, 1993.

[6] A. Jaonani, S. Sayadi, and M. Vanthournhout, "Potent fungi decolorization of Olive mill wastewater," Enzyme and Microbial Technology, vol. 33, pp. 802-809, 2003.

[7] S. Chakraborty, M.K. Purkait, S.D. Gupta, S. De, and J. K. Basu, "Nanofiltration of textile plant effluent for colour removal and reduction in COD," Separation Purification Technology, vol. 31, pp. 141-151, 2003.

[8] D. Wesenburg and S.N. Agathos "White rot fungi and their enzymes for treatment of industrial dye effluents". Biotechnology Advances, vol. 22, pp. 161-187, 2003.

[9] D. Wesenburg, F. Buchou, S.N. Agathos, "Degradation of dye containing textile effluent by the agaric white rot fungi *Citocybula dusenii*," Biotechnology Letters, vol. 24, pp. 989-993, 2002.

[10] A. Zille, and A. Rehorek, "Degradation of azo dyes by Trametes villosa laccase over long periods of oxidative conditions," Applied and Environmental Microbiology, vol. 71, pp. 6711-6718, 2005.

[11] Y.C. Toh, and Y.P. Ting, "Decolourization of azo dyes by white rot fungi (WRF) isolated in Singapore," Enzyme and Microbial Technology, vol. 33, pp. 569-575, 2003.

[12] M. Tien, and T.K. Kirk, "Lignin peroxidase of *Phanerochaete chrysosporium*," Science, vol. 221 pp. 661, 1983.

[13] K.V. Radha, A. Regupathi, T. Arunagiri and T. Murugesan, "Decolorization studies of synthetic dyes using *Phanerochaete chrysosporium* and their kinetics," Process Biochemistry, vol. 40, pp. 3337-3345, 2005.

[14] APHA, "Standard methods for the examination of water and wastewater," 19th edition, Washington, American public health association, American water works association and water environmental federation, 1995.

[15] J. Swamy, and J.A. Ramsay "Effects of glucose and NH4+ concentrations on sequential dye decolouration by *Tramates versicolor*", Enzyme and Microbial Technology, vol. 25, pp. 278-284, 1999.

[16] S. Linko, and R. Haapala, "A critical study of lignin peroxidase activity assay by veratryl alcohol oxidation," Biotechnology Techniques, vol. 7 pp. 75-80, 1993.

[17] G. Eremektar, S. Huseyin, and M. Sureyya, "Investigation of the relation between COD fractions and the toxicity in a textile finishing industry wastewater: Effect of preozonation," Desalination, vol. 211 (1-3), pp.314-320, 2007.

[18] S. Singh, and K. Pakshirajan, "Enzyme activities and decolourization of single and mixed azo dyes by the white-rot fungus *Phanerochaete chrysosporium*," International Biodeterioration and Biodegradation, vol. 64, pp. 146-150, 2010.

[19] M. Isik et al., "Effects of alkalinity and co-substrate on performance of an up flow anaerobic sludge blanket (UASB) reactor through decolorization of Congo Red Azo dye," Bioresource Technology, vol. 96 (5), pp. 633-643, 2003.

[20] R.G. Saratale, G.D. Saratale, D.C. Kalyani, J.S.Chang, S.P.Govindwar "Enhanced decolourization and biodegradation of textile azo dye scarlet R by using developed microbial consortium-G.R," Bioresource Technology, vol. 100 (9), 2009.

[21] S. Singh and K. Pakshirajan and A.Daverey "Enhanced decolourization of Direct Red 80 dye by the white rot fungus. *Phanerocheate chrysosporium* employing sequential design of experiments," Biodegradation, 21 (4), 501-511, 2010.

[22] A. Latif, S. Noor, Q.M. Sahrif, M. Najeebullaha "Different techniques recently used for the treatment of textile dyeing effluents: A review" Journal of Chemical Society of Pakistan, vol 32 (1), pp. 115-124, 2010.